

## Rapid Hippurate Hydrolysis Method for Presumptive Identification of Group B Streptococci

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A rapid test to detect the hydrolysis of sodium hippurate by beta-hemolytic streptococci within 2 h was developed. All group B streptococci tested were positive using this method and all other groups were negative.

From recent reports (2, 5) it appears that group B beta-hemolytic streptococci are frequently incriminated in severe infections including septicemia, meningitis, and pneumonia in the neonate and infant. It is important, therefore, for the clinical microbiology laboratory to be able to distinguish group B from other beta-hemolytic streptococci. The ability of group B streptococci to hydrolyze hippuric acid to benzoic acid and glycine was used as a definitive test before serological grouping of streptococci had been developed (1). The benzoic acid produced was detected by its precipitation in an excess of ferric chloride. Recently Facklam et al. (4) recommended that sodium hippurate hydrolysis be used for presumptive identification of group B streptococci; however, the method they cite requires 48 h.

The purpose of this study was to develop a rapid method for determining sodium hippurate hydrolysis by using a heavy inoculum of organisms in an aqueous sodium hippurate substrate and detecting the hydrolysis product, glycine, rather than benzoic acid. A 1% aqueous sodium hippurate solution was prepared and dispensed in 0.4-ml portions, capped or corked, and frozen at  $-20^{\circ}\text{C}$  until used. The tubes were thawed before inoculating, and a large loopful of beta-hemolytic streptococci from tryptic soy agar with 5% sheep blood (GIBCO) was emulsified in the substrate. The tubes were incubated for 2 h in a heating block at  $37^{\circ}\text{C}$ . Glycine was detected using a solution of ninhydrin (3.5 g of ninhydrin in 100 ml of a 1:1 mixture of acetone and butanol). After incubation, approximately 0.2 ml of the ninhydrin solution was added to the tubes. The tubes were returned to the heating block at  $37^{\circ}\text{C}$  for 10 min and then removed and observed. Organisms which were able to hydrolyze sodium hippurate produced a deep purple color with the ninhydrin reagent;

this color was as deep as crystal violet used in the Gram stain. Other beta-hemolytic streptococci showed no color reaction or occasionally a faint tinge of purple color was observed.

We tested 45 strains of beta-hemolytic group B streptococci and all were positive using this rapid method for detecting hippurate hydrolysis. Twenty-six of these organisms were stock strains and the remaining 19 strains were clinical isolates detected on primary isolation plates of specimens streaked and stabbed on tryptic soy agar with 5% sheep blood (GIBCO). Beta hemolysis of the stock strains and the clinical isolates was detectable in the area of streaking as well as in the stabs made in this medium. Other clinical isolates of beta-hemolytic streptococci tested were 28 group A, 7 group C, 1 group D, 8 group G, and 1 group F; all of the organisms in these groups were negative. A fluorescent antibody method was used to identify group A streptococci (3); the specific grouping of the other beta-hemolytic streptococci was performed in a separate streptococcal reference laboratory by the Lancefield technique as described by Frank and Levinson (6).

The usefulness of this rapid method for detecting hippurate hydrolysis by group B beta-hemolytic streptococci appears clear. It should be kept in mind, however, that not all group B streptococci are beta hemolytic (9). Also, it is well to remember that a small percentage of enterococci are beta hemolytic (4) and when this organism is suspected a rapid bile-esculin hydrolysis test (8) could be run in conjunction with this rapid hippurate hydrolysis method for the presumptive identification of this organism. Until high quality conjugated antiserum for group B streptococci is commercially available for the identification of group B streptococci by fluorescent microscopy (7), the rapid hippurate hydrolysis method described will be of assist-

ance in the rapid presumptive identification of group B beta-hemolytic streptococci.

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